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Review article

Assessment of nutritional interventions for modification of age-associated cognitive decline using a canine model of human aging

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Abstract

The present review focuses on the utility of a canine model in evaluating nutritional interventions for age-related cognitive dysfunction. Aged dogs demonstrate progressive cognitive decline with concurrent amyloid-beta pathology that parallels the pathology observed in aging humans. Dogs, therefore, provide a natural model of human pathological aging. We have and are in the process of evaluating several nutritional-based interventions aimed at preventing cognitive decline and brain aging. In a three-year longitudinal study, we examined the effects of a diet enriched with antioxidants and mitochondrial cofactors on several measures of cognition and brain aging. Compared to controls, aged dogs on the enriched diet demonstrated both short- and long-term cognitive benefits, as well decreased deposition of amyloid-beta protein. The diet also reduced behavioral signs associated with canine Cognitive Dysfunction Syndrome when assessed in veterinary clinical trials. We also have preliminary evidence suggesting a beneficial effect of a proprietary blend of docosahexaenoic acid and phospholipids on both cognitive and physiological measures. Collectively, our data indicate (1) that the dog, either in the laboratory or in the clinic, provides an important tool for assessing nutritional interventions and (2) that combination interventions aimed at several mechanisms of pathological aging may prove more effective than single nutritive components in human trials.

Abbreviations: Aβ – amyloid-beta; CDS – cognitive dysfunction syndrome; DHA – docosahexaenoic acid; DNMP – delayed-non-matching-to-position

Introduction

Advances in medicine and technology have resulted in an increase of human life-span over the last century. This increased longevity, however, is associated with an increased prevalence of age-related cognitive disorders, the most frequent being Alzheimer's disease. Given the impact of cognitive impairment on quality of life and cost to society, there is a pressing need for identifying interventions that can halt, reverse, or ideally prevent, progressive cognitive decline. One strategy for systematically evaluating interventions for, and mechanisms of, cognitive decline consists of animal models. The ideal animal model should demonstrate a decline in cognitive function with associated brain pathology consistent with that present in humans.

The most widely studied animal models, thus far, are rodents and nonhuman primates. More recently,

we have characterized a canine model of human aging that provides some unique features for evaluating interventions for age-related cognitive decline (Adams et al. 2000a). First, dogs develop age-related cognitive decline consistent with that reported in humans (Adams et al. 2000a). Second, humans and dogs show several parallels with respect to age-associated brain pathology, such as amyloid-beta (Aβ) deposition, ventricular enlargement and vascular changes (Cummings et al. 1996a). Third, in many respects the level of cognitive function seen in dogs is comparable to that in primates, and in the case of social cognition, the dog appears uniquely linked to humans (Hare et al. 2005). Last, dogs and humans have similar nutritional needs. Our work has facilitated the development of the canine model by linking cognitive changes that occur in canine aging with neuropathological changes. This justifies using the dog to evaluate nutritional interventions, and we have now examined several.

The present review addresses the utility of the canine model for evaluating nutritional interventions. We first discuss parallels between canine and human aging with respect to age-associated cognitive decline and neuropathology. We then highlight the effectiveness of a nutritional-based intervention consisting of a combination of antioxidants and mitochondrial cofactors. We also discuss preliminary studies using a proprietary blend of docosahexaenoic acid (DHA), an omega-3 polyunsaturated fatty acid, and an array of pig brain phospholipids.

Age-associated cognitive decline in dogs

We have used both cross-sectional and longitudinal studies to examine the effects of age on canine cognitive function. Our results indicate that cognitive function declines with age in dogs, but that the decline is domain, or task, specific and sensitive to previous experience. Procedural learning and memory, as well as simple discrimination learning, are generally insensitive to aging (Milgram et al. 1994). By contrast, tests of executive function, such as discrimination reversal learning (Milgram et al. 1994; Tapp et al. 2003a), and working memory (Adams et al. 2000b; Head et al. 1995), such as a delayed-nonmatching-to-position (DNMP) task (Chan et al. 2002), are highly sensitive to aging. In other instances, age sensitivity depends on prior test experience. Naïve young and aged dogs do not differ in their ability to learn relatively simple size discrimination tasks (Milgram et al. 1994). Once both groups have repeated experience on discrimination learning tasks, however, age differences emerge in that aged dogs require more trials to learn the task than do young dogs (Milgram 2003). As in other species, we have demonstrated that a treatment of environmental enrichment in the form of physical exercise, cognitive testing and kennel mates can improve learning ability in aged dogs (Milgram et al. 2004, 2005). Complex discrimination tasks, such as an oddity problem (where animals are required to respond to the different object of three for reward), are sensitive to age when objects are increasingly similar (Cotman et al. 2002). Thus domain-specific cognitive decline is apparent in canine aging and is modified by previous experience.

Using a cross-sectional approach, we have established that tasks dependent on frontal lobe function, in particular tests of executive function (Milgram et al. 1994; Tapp et al. 2003a) and visuospatial working memory (Chan et al. 2002), are notably sensitive to age (Adams et al. 2000b; Head et al. 1995). In a study of 109 dogs (manuscript in preparation), a statistically significant impairment in both acquisition of the DNMP using a 5-s delay and memory capacity were detected as early as six years of age (Figure 1) (Araujo 2004; Araujo et al. 2004a). Aged dogs also demonstrate increased variability in which some show little or no impairment (successful agers) while at the other extreme are those incapable of learning (demented) (Adams et al. 2000b). In aged dogs that perform equivalently to young dogs on the DNMP (successful agers), increasing memory load by adding an additional position results in a memory deficit (Tapp et al. 2003b). Longitudinal testing indicates a similar pattern, but also reveals marked decline in individual dogs that may span several cognitive domains (Adams et al. 2000a). These data are consistent with age-associated increases in individual cognitive performance in humans and with the hypothesis that age-associated cognitive dysfunction is a progressive process, such as the likely progression to Alzheimer's disease observed in patients diagnosed with mild cognitive impairment (Larrieu et al. 2002). Further, aged dogs demonstrate early deficits in executive function and visuospatial working memory, which are both early features of age-associated cognitive dysfunction in humans (Bartus 2000; Stuss et al. 1996; Flicker et al. 1984).

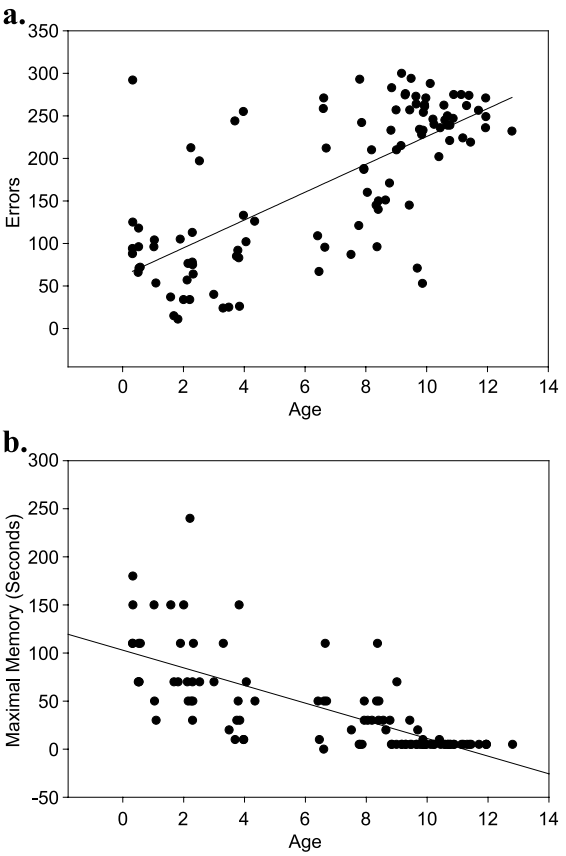

Figure 1. Errors to acquire the DNMP task at a 5-s delay (a) and memory capacity (b). In a study of 109 dogs, we divided the dogs into six age groups that included puppies (<1 year), young (1–2.99 years), adult (3–4.99 years), middle-aged (6–7.99 years), old (8–9.99 years), and senior (10–11.99 years). In addition to a significant effect of age [$P < 0.05$] on acquisition, the data indicated that middle-aged dogs were impaired significantly compared to both young and adult dogs. An identical effect was seen on memory capacity. This indicates that both acquisition of the DNMP and memory capacity are impaired early in canine cognitive decline.

Veterinarians also have identified an aging syndrome in pet dogs termed Cognitive Dysfunction Syndrome (CDS) (Landsberg and Ruehl 1997; Bain et al. 2001; Ruehl et al. 1995) that is typically diagnosed because of behavioral changes such as inappropriate house-soiling, alterations in activity levels, reduced interaction with family members, and wandering (Landsberg and Ruehl 1997). It is unclear to what extent CDS is related to neuropsychological deficits; however, evidence from laboratory-based noncognitive behavioral testing suggests a link. In particular, dogs impaired on neuropsychological tests are more likely to demonstrate increased activity levels compared to age-matched cognitively normal dogs (Siwak et al. 2001, 2003). Impaired dogs, when compared to young dogs or cognitively intact older dogs, spend less time close to or in contact with humans (Siwak et al. 2001). These results suggest that noncognitive behavioral changes also occur with severe cognitive impairment, which may partially model the noncognitive behavioral changes seen in human dementia.

The age-related behavioral impairments observed in the laboratory and the clinic likely represent a continuum in severity of cognitive decline. For example, service dogs, which are required to provide a high level of learned skills, may demonstrate age-related behavioral deficits at an earlier age than the average pet. While we have linked the cognitive deficits observed in the laboratory to pathological changes in the aging brain (see below), the behavioral signs associated with CDS are also likely due to a similar pathology (Cummings et al. 1996a, b; Landsberg and Ruehl 1997). Thus, nutritional interventions that reduce the development of brain pathology should have important benefits for pet dogs.

Brain aging in the dog

The aged canine brain exhibits several features that also occur in human pathological brain aging. In the early 1900s, abnormal pyramidal neuron sprouting was described (Lafora 1914). By the 1950s, researchers observed "Alzheimer's-like" plaque pathology (Dahme 1962, 1967, 1968; Osetowska 1966), which is a result of Aβ protein deposition (Cummings et al. 1996c). More recent studies have found that dogs have reduced levels of endogenous antioxidants (Head et al. 2002), cortical atrophy (Tapp et al. 2004a), ventricular enlargement (Tapp et al. 2004a; Su et al. 1998), myelin degeneration (Ferrer et al. 1993), and accumulation of degraded proteins (Borras et al. 1999). However, the canine and human neuropathology is not identical. Patients with Alzheimer's disease develop mature neurofibrillary tangles, which result from the intracellular accumulation of hyperphosphorylated tau protein. Dogs also show hyperphosphorylated tau, but mature neurofibrillary tangles are not seen (Wegiel et al. 1998; Papaioannou et al. 2001). Although the absence of mature neurofibrillary tangles limits the dog as a model for late

Table 1. Common features of Alzheimer's disease in the aged rodent and canine models.

Features of Alzheimer's disease	Rat	Dog
Aβ pathology	−	+
Aβ angiopathy	−	+
Neurofibrillary tangles	−	−
Markers of oxidative damage	+	+
Cortical atrophy	−	+
Ventricular enlargement	−	+
Memory deficits	+	+
Noncognitive behavioral alterations	+	+
Commercial application for interventions	−	+
Cognitive decline associated with Aβ pathology	−	+

+ Present; − absent.

stage Alzheimer's disease, the collective data indicate that canine brain aging models early stage pathology (Table 1).

Extensive work has been conducted to better understand the Aβ pathology observed in the aging dog. Aβ is naturally deposited in the canine brain with increasing age, forming diffuse plaques (Cummings et al. 1996b; Head et al. 1998, 2000; Satou et al. 1997). The predominant species of Aβ is the longer, more toxic, 42 amino acid protein, which is identical in sequence to that of the human (Cummings et al. 1996a,c). At later stages in the pathology, the shorter 40 amino acid fragment also accumulates in plaques and blood vessel walls as seen in Aβ angiopathy (Prior et al. 1996; Walker 1997). The distribution of plaques is region specific; the prefrontal cortex accumulates plaques more consistently and at an earlier age than the parietal, entorhinal and occipital cortices, which is consistent with the deposition pattern seen in humans (Head et al. 2000). Aβ accumulates first in the deeper cortical layers and subsequently in the superficial cortical layer (Satou et al. 1997). Unlike humans, Aβ accumulation is not observed in layer 1 of the canine cortex, but a diffuse band is observed in the outer molecular layer of the hippocampus in both species (Cotman et al. 2002).

Both cognitive dysfunction and cortical atrophy are linked to Aβ deposition in the dog (Tapp et al. 2004a; Cummings et al. 1996b). As mentioned previously, Aβ typically is deposited early in the prefrontal cortex of both dogs (Head et al. 2000) and humans (Braak and Braak 1997). Cognitive tasks thought to be dependent on the prefrontal cortex are impaired by age more consistently and earlier in the dog (Tapp et al. 2003a, b, 2004a, b). Further, the frontal lobes selectively atrophy in aged dogs prior to overall brain atrophy (Tapp et al. 2004a). This pattern of frontal lobe-based deficits is consistent with humans, where frontal lobe volume is correlated with impairment in executive function (Gunning-Dixon and Raz 2003) and is highly sensitive to age (Jernigan et al. 2001; Resnick et al. 2000).

Nutritional interventions in the aging dog

Antioxidants and mitochondrial cofactors

According to the oxidative stress hypothesis, aging is linked to an accumulation of oxidative damage caused in part by a decrease in mitochondrial function and a reduction of endogenous metabolic strategies to counteract the increase in oxidant species (Ames et al. 1993; Beal 1995; Shigenaga et al. 1994). The central nervous system is particularly sensitive to oxidative stress because of high metabolic rates and reduced antioxidant defenses compared to other tissues (Halliwell 1992). This may be responsible for cognitive decline and associated neuropathology (Beal 1995). To examine this hypothesis, we conducted a three-year longitudinal study in both young and aged dogs using two diets: (1) a diet enriched with a broad spectrum of antioxidants and mitochondrial cofactors and (2) an isocaloric control diet (Table 2). During the course of the study, cognition was monitored and half of the animals were sacrificed at study completion to determine the effects of the diet on brain pathology.

After treatment wash-in, we tested the subjects on an allocentric discrimination task, the landmark task (Milgram et al. 2002a), in which subjects were required to utilize the location of an external land-

Table 2. Antioxidants and mitochondrial cofactors included in the test diets.

Ingredient	Control diet (ppm)	Enriched diet (ppm)[a]
D,L-Alpha-tocopherol acetate	120	1,050
L-Carnitine	<20	260
D,L-alpha-lipoic acid	<20	128
Ascorbic acid as Stay-C	<30	80

[a]1% inclusion of each of the following was in the enriched diet (1:1 exchange for corn): spinach flakes, tomato pomace, grape pomace, carrot granules, and citrus pulp.
ppm: parts per million.

mark to determine the location of reward. The rationale for developing an allocentric test was based on the known propensity of Alzheimer's disease patients to become lost, even in familiar surroundings (Cogan 1979), thereby exhibiting deficits in allocentric spatial ability. Consequently, we hypothesized that performance on this task would be sensitive to aging and would be improved with the dietary intervention. For the landmark task, subjects were required to respond to one of two identical coasters based on the proximity of the coaster to an external landmark, a yellow peg, which could be placed at various distances from each coaster (Milgram et al. 1999). The further the landmark from the rewarded coaster, the more difficult the task. Aged dogs on the enriched diet learned the initial discrimination with fewer errors and on average were more likely to pass the learning criteria of subsequently more difficult levels than those on the control diet. By contrast, young dogs on the enriched diet did not differ significantly from young animals on the control food. We also examined remote memory for the landmark task by retesting the subjects approximately seven months later. Aged dogs showed poorer retention of the task compared to young dogs. There was, however, a strong tendency for aged dogs on the enriched diet to relearn the landmark task with fewer errors than aged animals on the control diet.

We also examined the effect of the diet on a set of complex discrimination learning problems using an oddity discrimination task (Cotman et al. 2002; Milgram et al. 2002b). In this task, the subjects were presented with three objects, two of which were identical and the other was the "odd" object. Dogs were required to approach and displace the odd object to obtain a food reward. Using a set of oddity problems, we were able to examine performance under various difficulty levels by using stimuli that were increasingly more similar. Thus, when the objects were more similar in appearance, dogs typically committed more errors to acquire the rule. Young dogs on the enriched diet did not differ compared to young dogs on the control diet at any difficulty level. We also did not see statistically significant effects of the diet on the easiest oddity discrimination problems in aged dogs. When the difficulty level was increased, however, aged dogs on the enriched diet learned the discrimination with fewer errors than the aged dogs on the control diet. The results of this experiment are important for several reasons. First, it demonstrates that the difficulty of a discrimination task is important when examining age differences, which may explain reports of both age-dependent and independent effects on discrimination learning and reversals in nonhuman primates (Voytko 1999). Second, it demonstrates that using multiple difficulty levels is more effective for assessing the effects of an intervention than a single discrimination task; a single discrimination task may not have revealed an effect if the difficulty level was either too easy or too difficult. Lastly, it demonstrates that the enriched diet is effective in attenuating age-related impairments in complex discrimination learning.

The effects of the diet on visuospatial memory and long-term retention using a DNMP task were examined also (manuscript in preparation) (Araujo et al. 2004b). We examined both relearning and memory capacity, defined as the longest progressive delay a dog could remember the location of an object within a standardized number of test sessions. The dogs were trained initially at baseline and were tested after one and two years on treatment. The subjects on both the control and enriched diet did not differ at baseline and after one year of treatment. By the second year of treatment, however, the subjects on the enriched diet demonstrated increased memory capacity (Figure 2) and improved relearning (Figure 3). Additionally, the pass frequency for the two groups differed only after two years such that the diet-enriched group passed the relearning criterion at over twice the frequency as the control group. These results suggest that the antioxidant treatment resulted in maintained cognitive function whereas the control group showed cognitive decline.

Finally, the longitudinal study assessed changes in discrimination and reversal learning (Milgram et al.

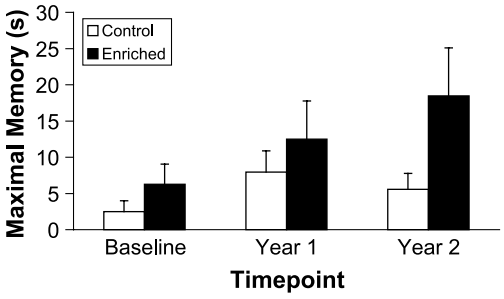

Figure 2. Memory capacity over three years. There was a trend for increasing memory capacity in dogs on the combination antioxidant and mitochondrial cofactor diet compared to dogs on the placebo diet. This difference was most apparent after the subjects had been on their respective treatment conditions for two years.

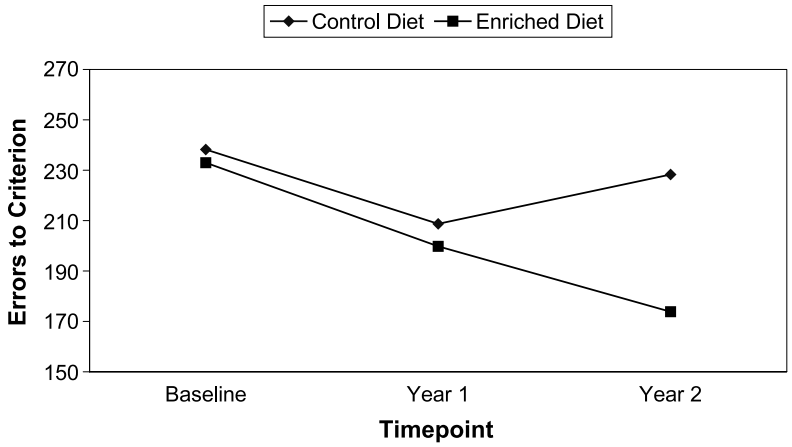

Figure 3. Long-term retention of the DNMP task at a 5-s delay. At baseline, the two groups of dogs did not differ from one another. Significant differences emerged two years following treatment such that the dogs on the antioxidant and mitochondrial cofactor combination diet reacquired the task with significantly [$P < 0.05$] fewer errors than the control group. The control group was performing no different than at baseline after two years on treatment.

2004, 2005). Dogs received a different discrimination and reversal problem at baseline, and after one and two years of treatment. As the study progressed, the enriched diet group maintained and even improved discrimination and reversal learning ability. By contrast, the control group showed deterioration in these cognitive abilities, especially reversal learning. The positive effects of the diet on reversal learning were even more robust when combined with environmental and cognitive enrichment. These experiments demonstrate that the effects of the enriched diet on certain cognitive tasks may only become apparent after several years of testing, possibly due to attenuation, or reduced rate, of progressive cognitive decline.

The diet was also examined in veterinary clinical trials and found to have beneficial effects on signs associated with CDS. To our knowledge, a cocktail intervention similar to the one used in our studies has not been examined in human aging. Although some positive results have been reported in clinical trials examining the effects of Vitamins E and C (Di Matteo and Esposito 2003) and antioxidant supplements (Grodstein et al. 2003; Jama et al. 1996; Engelhart et al. 2002; Helmer et al. 2003; Zandi et al. 2004) on several aspects of aging (McDaniel et al. 2003; Martin 2003; Martin et al. 2002), the studies reported here suggest that a cocktail intervention consisting of several antioxidants and mitochondrial cofactors may be more effective, possibly due to a synergistic effect. Similarly, studies in humans (Grodstein et al. 2003; Zandi et al. 2004; Masaki et al. 2000) and rodents (Joseph et al. 1998, 1999; Bickford et al. 2000) suggest that combination antioxidant therapy may be more effective than single component supplementation.

The results of the cognitive portion of the study indicate that a diet enriched with antioxidants and mitochondrial cofactors can improve cognition in aged dogs and likely attenuate the progressive nature of cognitive decline. This suggests that the diet may have both short- and long-term effects on brain pathology presumed to be responsible for cognitive impairment. To examine the effects of the diet on brain pathology, half the animals were sacrificed at the end of the study and their brains were harvested for pathological assessment [procedure described in Head et al. (2000)]. Overall, there was a significant reduction of Aβ load. Aβ loads were decreased in deep cortical layers by 27%, 75%, 69.5% and 84.1% in the frontal, entorhinal, parietal and occipital cortices, respectively (Head et al. 2004). Thus, the cognitive portion of the study not only predicted efficacy in the veterinary clinic, but also predicted an effect of the dietary intervention on brain aging. Further studies are being conducted to elucidate the mechanism by which the diet could be reducing Aβ accumulation.

Proprietary blend of docosahexaenoic acid and phospholipids

The rationale for studying DHA is based on the hypothesis that decreasing membrane fluidity con-

tributes to declining cognitive ability in aged subjects. As humans age, neuronal membranes may become more rigid and, as a result, are less able to release neurotransmitters and respond to chemical messengers (Sharma et al. 1993). DHA is an omega-3 polyunsaturated fatty acid found in membrane phospholipids and a deficiency in this fatty acid may play a role in Alzheimer's disease. A low intake of fish (Morris et al. 2003), the major dietary source of DHA, and low serum DHA levels (Kyle et al. 1999) are both linked to an increase in likelihood of developing Alzheimer's disease, whereas a high intake of fish is related to a decreased risk (Morris et al. 2003). Similarly, Alzheimer's patients and humans with mild cognitive impairment have lower plasma levels of DHA and a larger n-6/n-3 fatty acid ratio (Conquer et al. 2000). In Alzheimer's patients, cholestryl ester-DHA levels, a biomarker for DHA, is negatively correlated with the severity of dementia (Tully et al. 2003). DHA also is thought to possess antioxidant properties (Gamoh et al. 2001; Hashimoto et al. 2002); thus supplementation should serve to replace oxidized DHA present in phospholipids. Furthermore, DHA readily toggles between several conformations, thereby allowing the membrane phospholipids to adapt to the changes in receptor or ion channel conformations (Huber et al. 2002; Koenig et al. 1997). DHA also may have beneficial anti-inflammatory effects [reviewed in Horrocks and Yeo (1999)] and has been shown to have positive effects in rodent models of Alzheimer's disease (Hashimoto et al. 2002; Calon et al. 2004).

A pilot study (manuscript in preparation) examined the benefits of supplementing dogs with a proprietary blend of DHA and pig brain-derived phospholipids (provided by Nutramax Laboratories, Edgewood, MD). In this pilot study, 15 senior dogs (of at least 12 years of age) were administered either a placebo capsule or a capsule containing the proprietary blend of DHA and pig brain-derived phospholipids (including phosphatidylserine, phosphatidylethanolamine with plasmalogens, phosphatidylcholine, phosphatidylinositol, sphingomyelin, phosphatidic acid and other lysolipids). After approximately 100 days of prestudy treatment, the animals that survived were maintained on their respective treatments and tested on the DNMP. The results indicated a trend towards improved visuospatial memory in the treatment group (Figure 4); when compared to their cognitively matched control animal (used to ensure equivalent cognitive groups at baseline), the treatment animals had maximal memory scores that were on average 30 s longer in all but two of seven instances. We also monitored changes in the dogs' serum fatty acid profile. As expected, the dogs provided with the proprietary blend showed a dramatic increase in DHA levels [$P < 0.001$] and a corresponding decrease in docosapentaenoic acid levels [$P < 0.001$], which is the omega-6 polyunsaturated fatty acid equivalent of DHA that typically accumulates in the presence of a DHA deficiency. Analysis of canine brain changes in DHA levels is currently underway. Finally, the dogs on treatment developed fewer health-related problems than controls [chi-square, $P = 0.038$] (Table 3). These data support conducting a larger study to better characterize the supplement's ability to improve cognition and quality of life in aged dogs. In humans, fish oil trials are currently in progress (Clinicaltrials.gov 2004). Phospholipid trials in humans, particularly phosphatidylserine, have shown minimal benefits (Amaducci 1988).

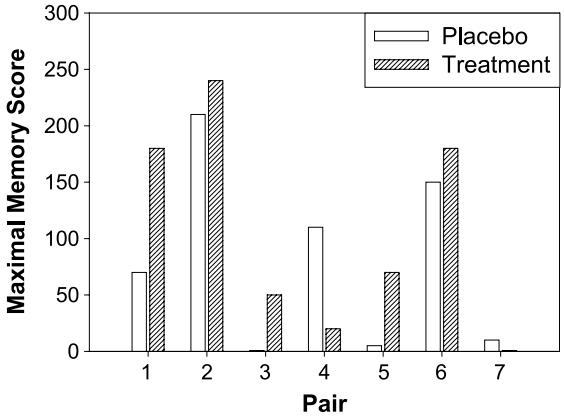

Figure 4. DNMP Maximal Memory Scores. Animals on treatment had a maximal memory score that was on average 30 s longer than their cognitively equivalent control animal. Higher scores were indicative of larger memory capacity.

Table 3. Health status of animals during treatment with either placebo capsule or proprietary blend of DHA and phospholipids.

Health status[a]	Placebo	Treatment
Healthy	3	7
Chronic illness	2	0
Euthanized	3	0

[a]The number of animals that were considered healthy, suffered from a chronic illness and that required euthanasia due to chronic illness are depicted.

Implications for assessment of nutritional interventions

The dog is a particularly useful model for studying nutritional interventions because dogs provide a natural model of progressive age-dependent cognitive decline with associated neuropathology that partially models the human condition. Alternative animals models include rodents and nonhuman primates. Because of their short life span and low cost, rodents are widely used in initial screening of cognitive-modifying interventions, but they are limited in their ability to model complex human cognitive abilities (Thomas 1996). Furthermore, aged rodents do not naturally model human Aβ neuropathology, although transgenic mice that overexpress APP and deposit Aβ are useful in this respect. Aged nonhuman primates are more suitable for studying age-associated cognitive decline than rodents, but are expensive to obtain, have long life spans, and can be difficult to work with compared to rodents and dogs. In addition, aged nonhuman primates predominantly express the shorter, less soluble, 40 amino acid species of Aβ (Gearing et al. 1996). Thus, the aged dog offers some advantages over other models, particularly for assessing interventions intended to prevent, attenuate, or reverse the cognitive decline associated with Aβ pathology (Table 1).

The importance of the present studies extends beyond their significance for human cognitive disorders; the studies also demonstrate that nutrition can affect aging and associated behavioral changes in companion animals. For example, a prescription diet based on the composition of the antioxidant and mitochondrial cofactor diet tested in our laboratory is now marketed for treatment of CDS (Prescription diet® canine b/d®). Furthermore, future studies of pet dogs may provide us with both retrospective and prospective data on the effects of nutrition on aging by monitoring conversion rates to CDS. These studies may prove particularly valuable because dogs typically are fed the same food daily, thereby reducing the variability inherent in human nutritional studies.

Our work is not the first to demonstrate that enriched diets can improve cognitive deficits in animals; positive results also are reported in rodent models (Joseph et al. 1998, 1999; Bickford et al. 2000). Nonetheless, the use of nutritional strategies remains controversial because of both positive (Grodstein et al. 2003; Jama et al. 1996; Engelhart et al. 2002; Helmer et al. 2003; Zandi et al. 2004; Martin 2003; Martin et al. 2002) and negative (Laurin et al. 2004; Lindeman et al. 2000; Luchsinger et al. 2003; Mendelsohn et al. 1998) results in human trials (McDaniel et al. 2003; Martin 2003; Martin et al. 2002; Mendelsohn et al. 1998). The findings in the longitudinal canine enriched-diet study are uniquely important because of the possibility that the combination of antioxidants and mitochondrial cofactors work synergistically, both by improving mitochondrial function and by compensating for the reduction in neuronal metabolic strategies for reducing the impact of free radicals. This combination resulted in both decreased Aβ deposition and improved cognitive status. Similarly, the DHA and phospholipids supplement targeted several potential mechanisms. Collectively, this data strongly suggests that future human trials may benefit from an increased focus on nutritional cocktails, as opposed to single nutrient supplementation. Although we are unaware of human studies using such a wide spectrum of antioxidants and mitochondrial cofactors, antioxidant combinations may be superior to single component supplements for human aging (Grodstein et al. 2003; Zandi et al. 2004; Masaki et al. 2000). Alternatively, the present results also may be due to nutritional limitations in the normal canine maintenance diet. In either instance, the results indicate that nutrition is an important factor in age-associated cognitive dysfunction. Considering that age-related cognitive impairment and approximately 95% of Alzheimer's disease cases are sporadic in nature, i.e., not attributable to genetic causes, a more thorough examination and appreciation of nutritional interventions is warranted.

Conclusion

The present review summarizes recent work in our laboratory on cognitive-modifying effects of long-term nutritional interventions in aged dogs. The dog demonstrates cognitive decline and brain pathology consistent with that observed in pathological cognitive decline in humans, such as that seen in early Alzheimer's disease. Therefore, the dog provides an alternative to rodent and nonhuman primate models for both screening interventions and examining mechanisms of pathological aging. We have found the canine model to be particularly useful for examining links among interventions, Aβ pathology,

and cognition. Thus far, we have examined or are in the process of studying several nutritional interventions, including antioxidants, mitochondrial cofactors, phospholipids, and DHA. The results suggest many or all of these may prove beneficial in both humans and pet dogs. One possibly important implication is that nutrition cocktails are likely to be more effective in human clinical trials than individual supplements. Collectively, the results of our studies indicate that dogs, both in the laboratory and likely as pets, provide a very powerful tool for examining the effects of nutritional interventions on age-associated cognitive dysfunction and related brain pathology.

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